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AUTHOR(S):

KITAKADO, YASUHITO; TANAKA, KOICHI; ASONUMA, KATSUHIRO; UEMOTO, SHINJI; MATSUOKA, SADAYUKI; UTSUNOMIYA, HIROFUMI; OKAMURA, RYUJI; ... OZAWA, KAZUE; SHIMAMOTO, TAKESHI; HYUN-SUONG

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A New Bioabsorbable Material for Rat Vascular Cuff Anastomosis: Establishment for the Long-Term Orthotopic Liver Transplantation Model

Yasuhito Kitakado, Koichi Tanaka, Katsuhiro Asonuma, Shinji Uemoto, Sadayuki Matsuoka, Hirofumi Utsunomiya, Ryuji Okamura, Tetsuo Katayama, Yukihiro Inomata, Kazue Ozawa, Takeshi Shimamoto*, Suong-Hyun*, Yoshito Ikada*

Second Department of Surgery, Faculty of Medicine, Kyoto University and *Research Center for Medical Polymers and Biomaterials, Kyoto University, 54 Kawaracho, Shogoin, Sakyoku, Kyoto, 606 Japan

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Abstract

Rat vascular anastomosis was performed using a newly synthesized bioabsorbable material (LA-GA copolymer) cuffs and the ordinary polyethylene cuffs. The LA-GA copolymer cuff which anastomosed the portal vein and the inferior vena cava were patent and developed no collateral veins even after 6 months, keeping the transplanted liver normal. By contrast, the polyethylene cuff anastomosed portal vein was completely occluded and the collateral veins were highly developed, with the transplanted liver showing the fatty degeneration of hepatocytes and numerous regenerative nodules. It is concluded that the LA-GA copolymer cuff is a suitable material for the short and long term study of rat orthotopic liver transplantation.

Introduction

Cuff vascular anastomosis is an excellent method that is widely used in orthotopic liver transplantation[1,2]. The polyethylene cuff employed in this method, however, has the disadvantages of foreign body reaction, fibrous tissue formation, incidence of immunological reaction and possible carcinogenesis after long-term use[3,4,5,6]. The result is that the venous anastomosis becomes occluded and the blood supply to the transplanted liver has to be maintained by collateral veins. So few studies have been done on the long-term orthotopic liver transplanted rats. It is necessary to establish a long-term orthotopic liver transplanted rat model.

At the Kyoto University Research Center for Medical Polymers and Biomaterials, a new bioabsorbable material (LA-GA copolymer) was synthesized from D,L-lactic acid and glycolic acid. This new bioabsorbable material has ester-bonds and can easily be hydrolyzed. The metabolites produced upon hydrolysis enter the metabolic pathways, and are excreted primarily as water and carbon dioxide. The rate of biodegradation is dependent on the molecular weight and composition ratio of

Key words: Inferior vena cava anastomosis, Portal vein anastomosis, Orthotopic liver transplantation, LA-GA copolymer cuff, Polyethylene cuff.
索引用語　下大静脈吻合，門脈吻合，同所性肝移植，LA-GA 共重合体カフ，ポリエチレンカフ
Present address: 2nd Department of Surgery, Faculty of Medicine, Kyoto University, 54 Shogoin, Kawaracho, Sakyoku, Kyoto, 606 Japan.

D,L-lactic acid and glycolic acid[7,8,9].

In this paper, the LA-GA copolymer cuff was used in the cuff-anastomosed rat inferior vena cava and in the orthotopic rat liver transplantation in order to examine the patency of the anastomosis and the histological change of the transplanted liver. The result was compared with the ordinary polyethylene cuffs.

Materials and Methods

Animal

Male Wistar rats weighing 300–380 g were used.

LA-GA copolymer

LA-GA copolymer was synthesized by the polycondensation of D,L-lactic acid and glycolic acid [7,8,9]. The copolymer has a molecular weight of 40–50 kDa, its composition ratio of D,L-lactic acid to glycolic acid is 8 : 2, and its *in vivo* degradation time is about 4 months. The cuffs used for the portal vein and inferior vena cava anastomosis had cuff bodies 2.0 mm and 2.5 mm long, 2.2 and 2.6 mm in outside diameter, and 2.0 and 2.4 mm in inside diameter, respectively.

Inferior vena cava anastomosis

Inferior vena cava graft procurement: Under ether anesthesia, the abdominal cavity was opened, and the right renal vessels and lumbar vein were ligated and dissected. The inferior vena cava was removed from the lower liver margin to the left renal vein for venous grafting. Cuff preparation was performed in a 4°C iced saline bath. Both ends of the vessel were everted over the cuff and secured with a circumferential 7–0 nylon suture.

Cuff technique for inferior vena cava anastomosis: Under ether anesthesia, abdominal cavity was opened, and a right kidney was resected. The inferior vena cava was cross-clamped with a mosquito clip, transecting the midpoint between the left renal vein and the lower liver margin. Cuff anastomosis was completed and secured with a circumferential 7–0 nylon suture. Rats were sacrificed at 3, 5, 7, 9, 11, 13, 15 and 17 weeks, both for the LA-GA copolymer and the polyethylene cuff.

Orthotopic liver transplantation

Orthotopic liver transplants were performed in male Wistar rats, using the modified cuff technique described by KAMADA *et al.*[1,2]. Recipients were divided into the LA-GA copolymer and the polyethylene cuff groups. Rats were maintained on a diet of laboratory chow and water *ad libitum* postoperatively.

Venography

The patency of the anastomosed inferior vena cava and portal vein, and formation of collateral veins were examined by venography, which was performed as follows. Under ether anesthesia, a silicon catheter was inserted into the femoral vein or the branch of the superior mesenteric vein through which contrast material (5 to 10 ml of 60% urograffin) was injected and X-ray films were then taken.

Light microscopy

Immediately after venography, 10 to 20 ml of physiological saline followed by 10% formaldehyde was perfused through the same catheter used for venography. The anastomosed inferior vena cava and portal vein and the transplanted liver were dissected free, removed, and processed for histology. The sections were prepared with hematoxylin and eosin.

Results

Inferior vena cava anastomosis with the LA-GA copolymer cuff

23 rats were anastomosed with the LA-GA copolymer cuff. All survived before sacrifice. In 3 rats, at 7, 11, 15 week, respectively, stenosis of the venous graft and formation of collateral veins were seen not because of the LA-GA copolymer itself but because of technical error, possibly due to the twisting of the venous graft. But tissue reaction such as granulation and fibrosis was absent and thrombus formation was not noted. Venography showed that neither stenosis nor occlusion were observed in the other 20 rats (Fig. 1A). The LA-GA copolymer cuff showed signs of being gradually absorbed, the time course for which is shown in Fig. 2. The LA-GA copolymer cuff became white-colored, softened and slightly swollen at 5 weeks and almost completely absorbed at 15 weeks. Microscopically, the LA-GA copolymer cuff was gradually reduced in thickness and completely disappeared at 17 weeks (Fig. 3A).

Inferior vena cava anastomosis with the polyethylene cuff

12 rats were anastomosed with polyethylene cuffs. All survived before sacrifice. Venous anastomosis gradually became stenotic and was occluded after 9 weeks. When the anastomosed vessels were occluded, collateral veins highly developed (Fig. 1B). The polyethylene cuff came to be encapsulated by granulation and fibrosis. Microscopically, foreign body reaction to the polyethylene cuff and thrombus formation in the anastomosed vessel were seen (Fig. 3B).

Orthotopic liver transplantation with the LA-GA copolymer cuff

Orthotopic liver transplantation was performed with LA-GA copolymer cuffs in 5 rats. All rats

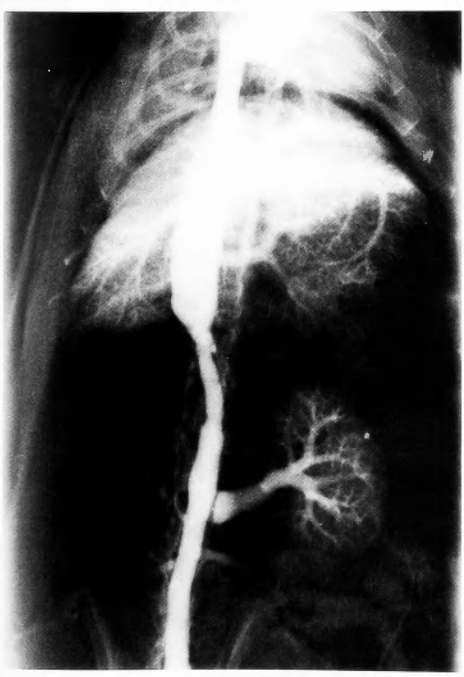

Fig. 1A Venography of the LA-GA copolymer cuff anastomosed inferior vena cava at 17 weeks. Neither stenosis nor formation of collateral veins were seen.

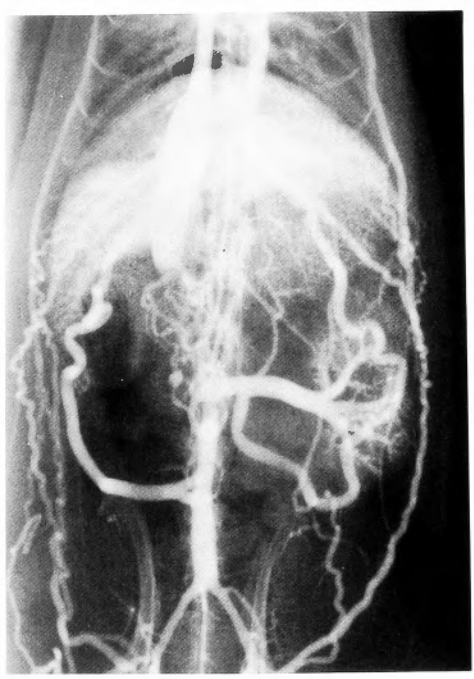

Fig. 1B Venography of the polyethylene cuff anastomosed inferior vena cava at 15 weeks. Note many collateral veins and occlusion of the anastomosis.

survived until sacrificed. Macroscopically, the LA-GA copolymer cuffs had completely disappeared from the anastomotic site after 6 months. Venography of the anastomosed portal vein showed neither stenosis nor collateral vein formation (Fig. 4A). Microscopically, foreign-body reaction and thrombus formation around the anastomotic site were not observed. Histology revealed that the transplanted liver was normal. Neither hepatocyte damage nor fibrosis were observed (Fig. 5A).

Orthotopic liver transplantation with the polyethylene cuff

Orthotopic liver transplantation was performed with polyethylene cuffs in 5 rats. The polyethylene cuff anastomosed portal veins were occluded and collateral veins were highly developed

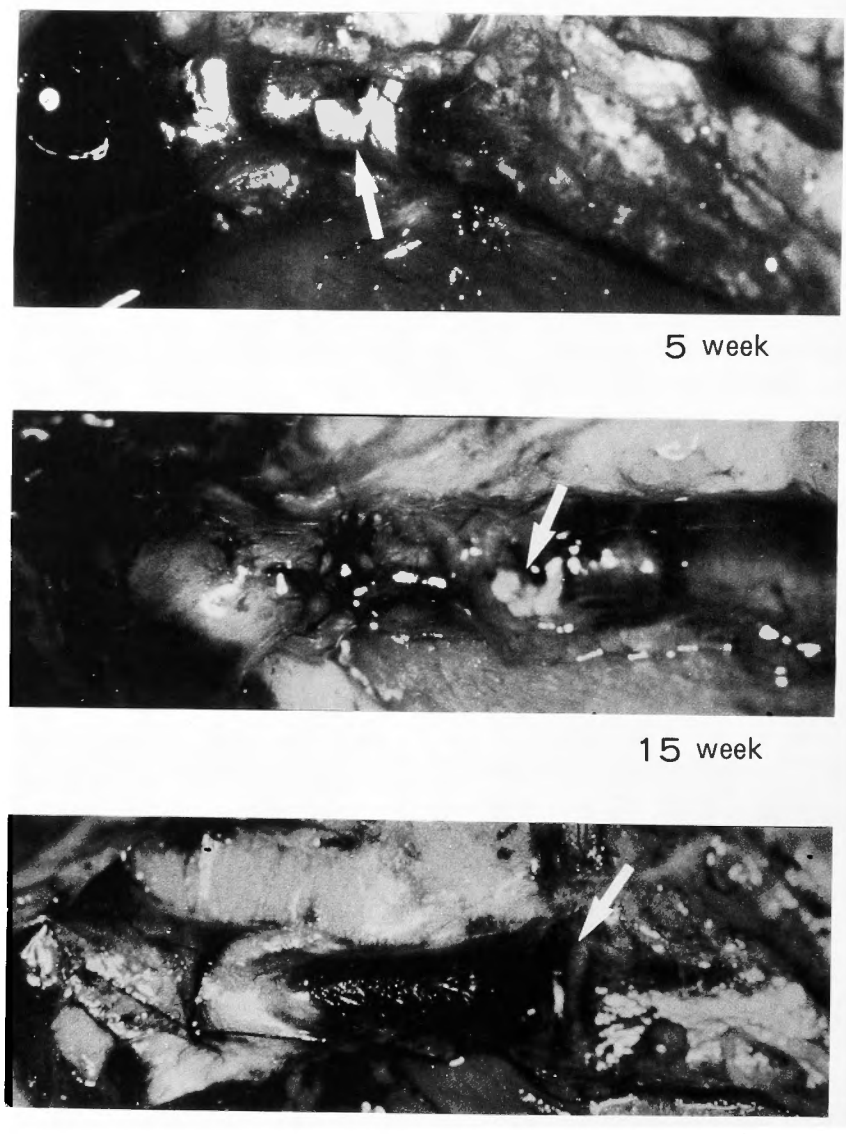

Fig. 2 Time course of biodegradation of the LA-GA copolymer cuff. At 5 weeks, it became white-colored, softened and slightly swollen. At 17 weeks, it was completely absorbed. The arrow shows LA-GA copolymer cuff.

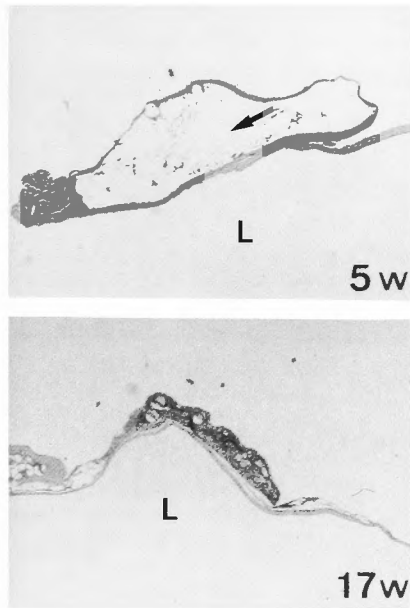

Fig. 3A Histology of LA-GA copolymer cuff anastomosis. Note that there is little foreign body reaction and no thrombus formation in the lumen. At 5 weeks, the LA-GA copolymer cuff (arrow) is the lucent area due to acetone treatment. At 17 weeks, it is no longer seen (20×, H & E). L=intravascular lumen

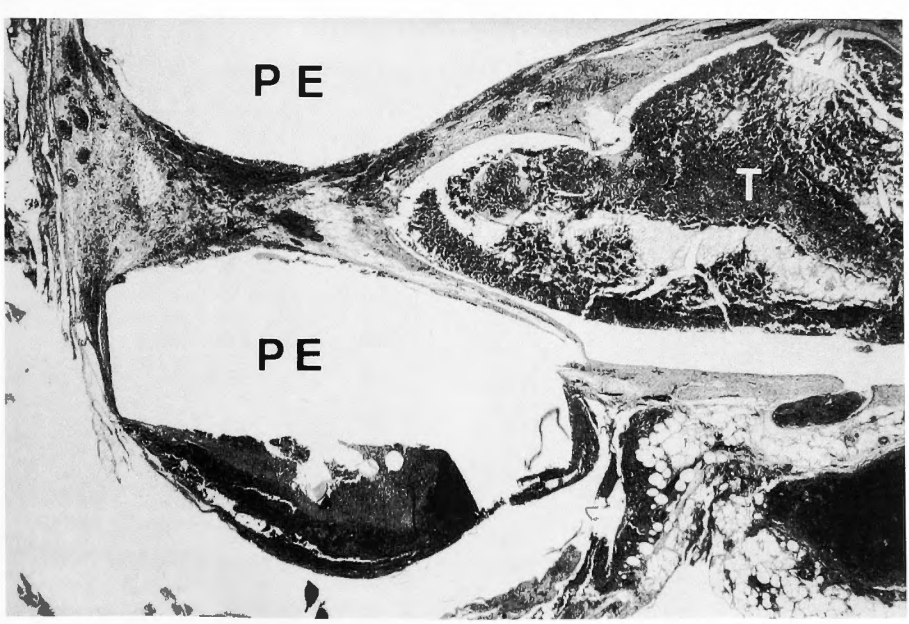

Fig. 3B Histology of polyethylene cuff anastomosis at 15 weeks. Note fibrosis around the cuff and thrombus formation in the lumen (20×, H & E). T=thrombus, PE=polyethylene

Fig. 4A Venography of the LA-GA copolymer cuff-anastomosed portal vein. Note there is neither stenosis nor collateral vein formation.

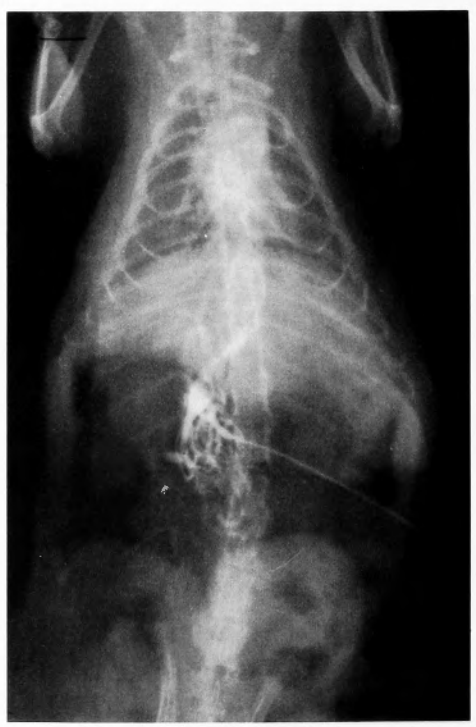

Fig. 4B Venography of the polyethylene cuff-anastomosed portal vein. Portal vein was occluded and collateral veins were highly developed.

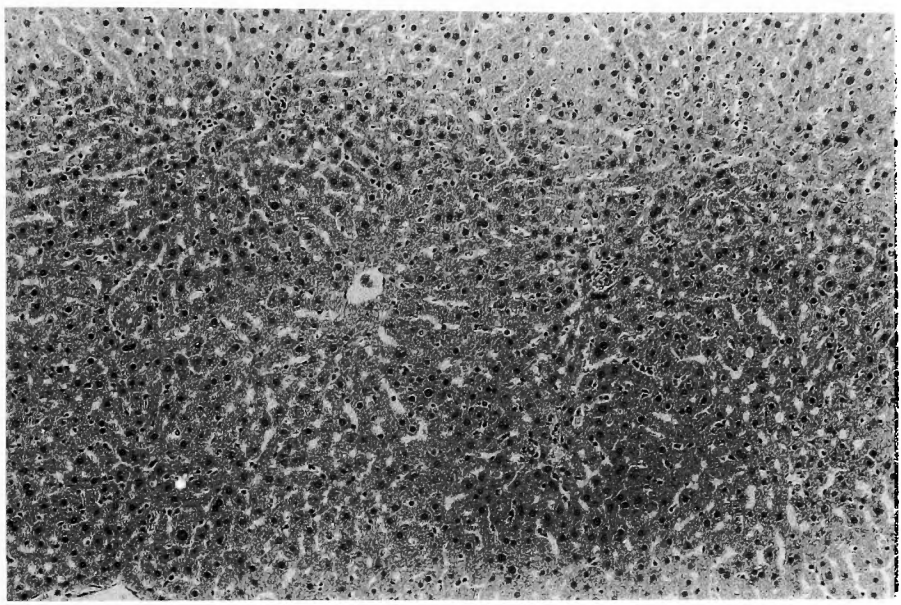

Fig. 5A Histology of the transplanted liver with the LA-GA copolymer cuff anastomosis. Note the absence of hepatocyte damage (200×, H & E).

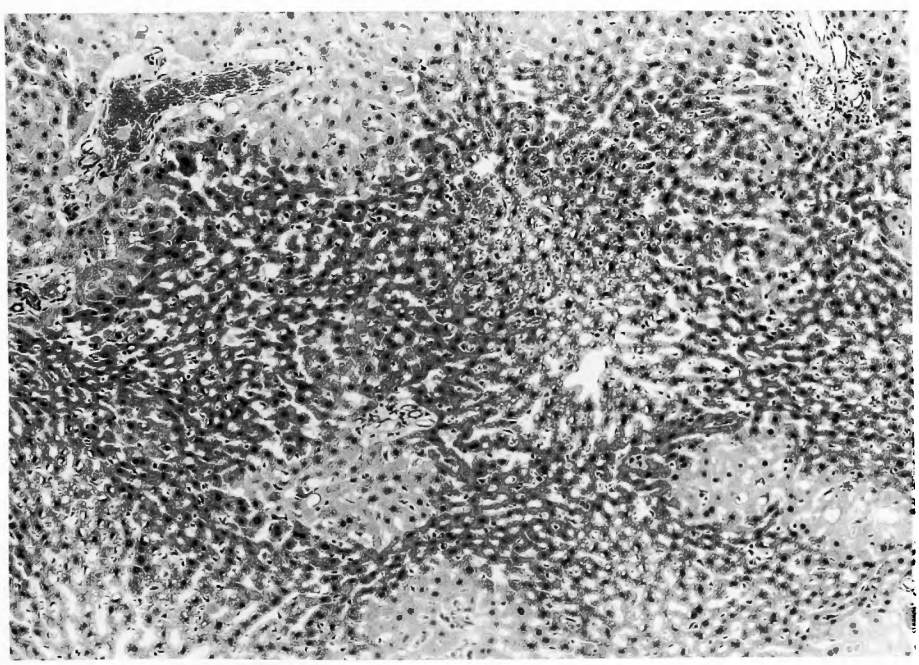

Fig. 5B Histology of the transplanted liver with the polyethylene cuff anastomosis. There was the fatty degeneration of the hepatocytes as well as numerous regenerative nodules (200×, H & E).

in all rats after 6 months (Fig. 4B). Foreign body reaction to the polyethylene cuff and thrombus formation in the anastomosed vessels were observed microscopically. Histology of the transplanted liver showed the fatty degeneration of hepatocytes and numerous regenerative nodules (Fig. 5B).

Discussion

As our previous report and those of others[10,11,12,13] reveal, the LA-GA copolymer cuff has certain characteristic advantages for vascular anastomosis. Basically, the copolymer disappears from the rat tissues within 4 months and produces little foreign body reaction. As a result, the anastomotic site is neither occluded nor constricted by the presence of the copolymer.

Iwasa *et al.* have performed rat microvascular anastomosis between the carotid artery and jugular vein using the LA-GA copolymer as an external splint and have confirmed the good patency and virtual absence of the foreign body reaction upon examination by angiography and microscopy[10]. But the arterio-venous anastomosis is different from veno-venous anastomosis in certain respects, such as blood pressure and flow. In rat orthotopic liver transplantation, veno-venous anastomosis is important because the transplanted liver is usually not being supplied with blood from the hepatic artery. Various methods for venous anastomosis have been proposed. Among them, the cuff method is widely used because it is relatively easy to perform. Many researchers have reported on the long-term survival of rat orthotopic liver transplantation using the polyethylene cuff, but few studies have been done on the long-term orthotopic liver transplanted rat because of the disadvantages of the polyethylene cuff employed in the anastomosis between portal vein and vena cava. Our histological findings revealed that anastomosed vessels with the polyethylene cuff became gradually stenotic and were finally occluded and collateral veins were highly developed. The foreign body reaction to the polyethylene cuff led to the stenotic change of the cuff-anastomosed vessel lumen. The stenotic change resulted in the blood flow stasis and thrombus formation. Histological study of these transplanted livers showed liver cell damage which was probably due to the portal vein obstruction. Although Kamada *et al.* described that the histology of the liver graft 438 days after liver transplantation showed preservation of normal lobular architecture, they did not state how many of the orthotopic liver transplanted rats kept normal liver architecture and the patency of the cuff-anastomosed portal vein. In our laboratory, a half of the orthotopic liver transplanted rats with polyethylene cuffs restored from this liver cell damage at the time of one year after liver transplantation probably due to the highly developed collateral veins.

On the other hand, the LA-GA copolymer cuff-anastomosed inferior vena cava and portal vein were patent and no collateral veins were developed even after 6 months. The histology of the transplanted liver was kept normal.

It is concluded that the polyethylene cuff is material suitable only for short term rat orthotopic liver transplantation. Using the LA-GA copolymer cuff, however, it is possible to perform either short-term or long-term rat orthotopic liver transplantation.

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和文抄録

新しい生体吸収材料カフによるラットの血管吻合
—長期肝移植モデルの確立—

京都大学医学部第二外科学講座
北角　泰人，田中　紘一，阿曽沼克弘，上本　伸二，松岡　貞幸
宇都宮裕文，岡村　隆仁，片山　哲夫，猪股裕紀洋，小澤　和恵

京都大学生体医療工学研究センター
島本　偉志，玄　　丞休，筏　義人

新しく合成された生体吸収材料（LA-GA 共重合体）カフを用い，ラットの血管吻合を行ない，従来のポリエチレンカフと比較検討した．LA-GA 共重合体カフで吻合した門脈，下大静脈は6ヶ月後でも側副血行路は認められずに開存し，移植肝は正常の組織像を保っていた．一方，ポリエチレンカフで吻合した門脈，下大静脈は完全に閉塞し，高度に発達した側副血行路が認められ，その結果移植肝には肝細胞の脂肪変性と多数の再生結節が認められた．以上の結果より，LA-GA 共重合体カフはラット同所性肝移植の短期及び長期の研究に適した生体材料だと結論付けられる．